

EVALUATING VARIATION IN
TERRESTRIAL PLANT TOXICITY TESTS

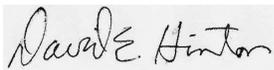
Master's Project

by

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Abstract

Purpose

The Office of Pesticide Programs of the U.S. Environmental Protection Agency (U.S. EPA) conducts the pesticide review process, under authority granted by the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Ecological risk assessments are performed on many different organisms to understand the ecosystem-level impact of a given pesticide. Among these organisms are non-target terrestrial plants, which are specifically used to understand the risk to plants exposed to spray drift or runoff from sites of intended application. Due to the development of guidelines from field-based methods rather than a traditional interlaboratory precision analysis, the natural observable variation in measurement endpoints for terrestrial plant toxicity tests has not been established. The following report aims to determine the natural variability of the response endpoints for these studies.

General experimental design

This project involved the development and design of two databases in MS Access, the selection of control data from existing vegetative vigor and seedling emergence studies, an analysis of the among- and within-laboratory variability in response measures, and a trend analysis of the data for variation due to environmental conditions.

Results

The findings of the descriptive statistics supported the currently accepted coefficient of variation (CV) of 20% used by the U.S. EPA. For the Seedling Emergence database, all studies in all laboratories had average CV values of less than 40% and laboratories responsible for conducting a majority of the studies (defined as 8 or more studies) exhibited even less variation with CVs less than 20%. Similar patterns were observed in the length data found in the Vegetative Vigor database. The weight data from the VV database presented variation that was often greater than the expected 20-30% even for the most frequently tested species. Among the species, the most data were available for corn and soybean and resultant data for these species were the least variable.

Conclusion

The historical value of a 20% coefficient of valuation seems to be, on average, an acceptable value for the terrestrial plant toxicity tests the U.S. EPA reviews for pesticide registration. The data of this report indicates that CVs for particular species, specifically corn and soybean, are lower, approximating values closer to 10%. The addition of more data and a more sophisticated analysis into the underlying causes of the variation that are evident in this initial data collection could reveal a lesser CV across all species.

Significance

By requiring CVs less than 20%, the EPA adds more precision and requires stricter pesticide assessments. Recognizing the inherent sensitivity of the assessments they are currently conducting, regardless of whether specific existing guidelines are changed, is important for the U.S. EPA to be able to better understand the pesticide review process.

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Introduction

Under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*), the U.S. Environmental Protection Agency (U.S. EPA) is granted the authority to regulate the use of pesticides. A formal review and registration process is conducted by the EPA on all pesticides approved for use in the United States.¹ The U.S. EPA Office of Pesticide Programs (OPP) currently conducts this review process, as authorized by the federal government under 40 CFR Parts 150 and 152.1.

In the pesticide review process, OPP is allowed to request submission of pesticide data from the applicant (40 CFR Part 158). Among other uses within OPP, the data is used in part to conduct risk assessments which evaluate both human and ecological health hazards and risks of pesticide use. The human health risk assessments obviously involve only one species, ourselves as humans. Conversely, ecological risk assessments span a wide array of species so as to assess the effect of pesticides on all the inhabitants of an ecosystem. Therefore, data on many different organisms is included in the ecological risk assessments to fully understand the effects on the ecosystem as a whole. Among these organisms are non-target terrestrial plants, defined as plants growing in terrestrial as opposed to aquatic environments. These tests are specifically used to understand the hazard or risk implications of pesticide use on non-target plant species exposed to spray drift or runoff. The requirements for non-target plant species toxicity tests are laid out by 40 CFR Part 158.590, which states the need for data on seedling emergence and vegetative vigor for terrestrial plants, and aquatic plant growth patterns.²

When reviewing a conventional pesticide which is used outdoors, the U.S. EPA looks for non-target plant toxicity tests. These tests focus on two life stages during plant development: early growth, involving seed germination through seedling emergence, and maturation, such as is covered in vegetation vigor tests. Seedling emergence studies encompass the early growth stages of the plants. Endpoints can include percent emergence, number of emerged plants, seedling survival, seedling height, and seedling biomass, as well as some qualitative phytotoxic effects.^{3,4} Studies of vegetative vigor focus on life stages occurring post-emergence and measure endpoints including survival, plant height, and plant biomass.^{5,6}

An important piece of the risk assessment is the fact the U.S. EPA itself does not conduct the non-target plant toxicity tests. The data is produced and collected by external, independent

¹ U.S. Congress. 1972. *Federal Insecticide, Fungicide, and Rodenticide Act*. 7 U.S.C. 136, *et seq.*

² U.S. 40 CFR Part 158.590.

³ U.S. EPA. 1996. Ecological Effects Test Guidelines. OPPTS 850.4100: Terrestrial Plant Toxicity Tier I (Seedling Emergence). EPA 712-C-96-153, April *Public Draft*.

http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Drafts/

⁴ U.S. EPA. 1996. Ecological Effects Test Guidelines. OPPTS 850.4225: Seedling Emergence Tier II. EPA 712-C-96-363, April, *Public Draft*

http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Drafts/

⁵ U.S. EPA. Ecological Effects Test Guidelines. OPPTS 850.4150: Terrestrial Plant Toxicity Tier I (Vegetative Vigor). EPA 712-C-96-163, April, *Public Draft*.

http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Drafts/

⁶ U.S. EPA. 1996. Ecological Effects Test Guidelines. OPPTS 850.4250: Vegetative Vigor Tier II. EPA 712-C-96-364, April, *Public Draft*

http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Drafts/

laboratories, which are generally the laboratories who have developed the pesticide, also known as the registrants. Registrants must get their pesticides approved for conventional use through the U.S. EPA review process; therefore it is in their best interest to submit this data in a timely manner to the Agency.

Even when identical methods are employed, unavoidable, random errors exist in scientific experiments, thus creating inherent variation in observed results. In general, before a test protocol is standardized, a “round-robin”, or interlaboratory, test is conducted to determine the precision of the test method.⁷ This round-robin test involves a predetermined test protocol which the participating laboratories must follow. Laboratories conduct the tests, and the data is analyzed for reproducibility and consistency, which is essentially validity, of the test measurement endpoints among and within laboratories.⁸ Additionally, the data reveal insight about the natural variation in the specific response measure (i.e. height, weight) of the species of interest under a set of given test conditions. Using the interlaboratory study results, specific test protocols can be further refined, such as the number of replicates used or the number of test organisms per experimental unit, to produce improved data for analysis.

Test protocols, however, can be developed without conducting round-robin or interlaboratory precision studies; instead, protocols arise from testing methods used in the field of study. Such is the case for the seedling emergence and vegetative vigor toxicity test guidelines as employed by the U.S. EPA. The lack of a round-robin or interlaboratory study has led to two important informational gaps in current terrestrial plant toxicity testing. These are described below.

First, in the absence of traditional interlaboratory study, the *natural observable variation* in measurement endpoints for vegetative vigor and seedling emergence tests is unknown. Without understanding the natural variation of results, the sensitivity of the tests cannot be fully established. The current test protocols use a coefficient of variation (CV), a ratio of the standard deviation to the mean, of 20% or less for both seedling emergence and vegetative vigor tests to determine the scientific soundness of tests conducted by laboratories. The magnitude of this CV, which is used for all response measures, was set based on expert opinion and practical concerns. Up to this point, this estimated CV has been sufficient, yet the internal and external validity of U.S. EPA risk assessment procedures could be greatly improved if an evidence- or results-based estimate of the natural variation of terrestrial plants was established.

Secondly, the protocol for conducting terrestrial plant toxicity tests allows for results produced under a broad spectrum of environmental conditions. Thus, data from applicant laboratories varies greatly in how the data was produced, for example in greenhouses or outdoors, with natural light, fluorescent supplementary light, or supplementary fluorescent and incandescent light, different post sizes, or with varied watering patterns from above or from below. The effect of the various growing conditions on the variability of the response measures is unknown. Therefore, the endpoint measurements generated by a given laboratory, in a given test under set conditions, may not be comparable to the data obtained from the same test done at another

⁷ ASTM International. 1999. Standard Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method. E 691-99.

⁸ Balls, M et al. 1995. Practical Aspects of the Validation of Toxicity Test Procedures. *ATLAI*. 23: 129-147.

laboratory or even another test done at the same laboratory under different environmental conditions (e.g., change in pot size, lighting schemes).

Under FIFRA, the toxicity tests must be conducted using good laboratory practice (GLP) standards.⁹ GLP standards ensure good documentation procedures and laboratory maintenance, but do not specify a specific protocol that must be followed. The recommended terrestrial plant guidelines include broad environmental conditions rather than a set protocol. A stringent protocol may not be necessary for vegetative vigor and seedling emergence tests, as it is assumed this range of environmental conditions does not introduce a high level of variability to the testing results. Furthermore, a review of the broad environmental conditions implemented in these tests and their impacts on inter- and intralaboratory variability could benefit U.S. EPA. The additional information could grant U.S. EPA insight into what conditions produce the most consistent results, or under what conditions create more variability, and thereby provide information to modify test protocols appropriately.

Objective

The following assessment sets out to determine the natural variability of the response endpoints among and within laboratories for vegetative vigor and seedling emergence studies by analyzing control data from studies submitted to the U.S. EPA. This analysis will help document the fundamental quality or precision of the terrestrial plant toxicity test guidelines, which has yet to be performed. The capability of most plant testing facilities to achieve a 20% CV using the current test guidelines can be evaluated and documented, thus allowing the U.S. EPA to adjust test guidelines, if needed, to acquire sufficient and accurate data for the review process.

Additionally, this study compiles data regarding the manner (e.g., pot size, lighting source and quality) in which the tests are conducted. Given the broad array of varied growth conditions, emergent trends and test conditions that minimize variability and promote the greatest plant growth, in terrestrial plant toxicity tests, can be uncovered. This should help document the need, if any, to establish more uniform testing protocols.

⁹ U.S. 40 CFR Part 160.

Methods

This project includes the development and design of two databases, the selection of as robust a sample as possible of control data from existing vegetative vigor and seedling emergence studies, an analysis of the among- and within-laboratory variability in response measures, and a trend analysis of the data to variation due to environmental conditions. An additional goal of the study is to highlight a set of recommendations for laboratories conducting these studies to ensure optimal growth conditions.

There is a wealth of seedling emergence and vegetative vigor control data available to the U.S. EPA from studies submitted to support pesticide registration. However, this large database was culled to a manageable size, using only the most appropriate data as indicated below, for this assessment. Proprietary or classified business information was removed in the case of analyses conducted independent of the U.S. EPA.

The vegetative vigor (VV) database was created in the summer of 2006, and a similar protocol was followed in the development of the seedling emergence (SE) database in 2007. The database framework was first created as a nested model in Microsoft Access. The data to be input into the database was selected from the control data available to the U.S. EPA. Studies selected were based on a number of parameters. Ideally, the database for assessing between and within laboratory analysis would include 10 studies, from 10 laboratories, which test the same 10 species. However, given the test guidelines these conditions are not possible. Test guidelines specify that 10 species be tested. A few species, in particular soybean, corn, and a root crop, are the only required tests; otherwise testing facilities have the freedom to select any combination of other species to make up the 10 per study. Therefore, to achieve the most data possible from all submitted studies with the greatest efficiency, the selection of studies for the VV and SE databases was limited to laboratories, and studies within those laboratories, which tested as many of the standard and “popular” species as possible. The goal of study selection was to approximate the ideal lab database (10 labs, 10 studies, 10 species). After the database was completed, the extent to which environmental test conditions differ should be evaluated to determine if additional data entry would be warranted to aid in evaluation of these factors for their contribution to variability.

After selection, the data was input into the Access database. An integral component to U.S. EPA use of data is quality assurance protocols to ensure reliability and validity of the database. The Quality Control Plan (QCP) for the VV database was created in the summer of 2006 and a similar approach was developed for the SE database. The QCP is a multi-step process that integrates checking of various sources to verify the integrity of the data. In some instances, a 100% check of the data entries is possible while in others 5% of the dataset is chosen for review due to the large number of records per table. The 5% will be selected using a random number generating program to create an unbiased subset of the data for analysis. Any errors found shall be corrected in the database immediately and documented on reviewer sheets. The error rate is determined by dividing the number of entries (not fields) containing errors by the total number of

entries checked.¹⁰ If the QC returns an error rate greater than 1%, the errors are to be corrected and the test is repeated. The test is to be carried out on another 5% from the remaining dataset and checked for errors until an error rate of less than 1% is achieved.

Once the database was completed and checked for errors, the dataset was analyzed to first determine descriptive statistics of within and between laboratory variability followed by descriptive statistics of the testing conditions. After these first analyses are undertaken, more sophisticated analyses can be undertaken to better establish trends for VV and SE tests separately. After establishing trends, the overall natural variability of growth in terrestrial plant toxicity tests can be found. Additionally, descriptive statistics of the environmental and growing conditions employed in the tests were developed, to aid U.S. EPA in developing future guidelines for laboratory protocol.

Materials

- Control data on the seedling emergence results from various studies from multiple laboratories, selected per the process described above.
- Environmental conditions data on those studies.
- Other basic study information.
- Statistical package to conduct rudimentary and advanced statistical analyses, in this instance MS Excel and the Excel Data Analysis Add-In.

¹⁰ An entry is a single row of data containing multiple fields. For example, in the Chemical Name Table one entry is a chemical name and its associated CAS# and PC Code fields. If one of those fields is found to have an error, the whole entry is counted as faulty when determining the entry level error rate.

Results and Observations

Preliminary descriptive statistical analyses on the two datasets reveal some new insight into the amount of variation detectable in terrestrial plant toxicity tests. The statistical analysis which follows focuses on specific endpoints from the seedling emergence (SE) and vegetative vigor (VV) tests. The chosen endpoint for the SE database is the classification of “emergence”, which is a binary descriptor for the seedling (i.e., it is emerged or it is not at the time of observation). Seedling length and dry weight are the selected endpoints for the VV studies. SE observations were made on multiple occasions for the same replicate throughout each study, for instance on days 7, 14, and 21. For standardization purposes, data recorded on day 14 is being used in this analysis. The VV endpoint measurements were taken at the termination of the study, generally at 14 or 21 days depending on the duration of the study.

When initially examining the data from both the SE and VV databases, the quantity of data is overwhelming and simultaneously inadequate. As previously mentioned, the target for these datasets was 100 studies covering 10 species in 10 laboratories. Given time constraints and data availability, the two datasets ended up with 58 studies in the SE dataset and 69 studies in the VV dataset. Additionally, the freedom granted to the laboratories to decide what species to examine, aside from corn, soybean, and a root crop, creates a wide array of unique or infrequently used species across the laboratories. The full lists of species recorded for each dataset can be found in Appendix (3). For the purposes of the following analysis, however, the species analyzed have been reduced to four based on the number of replicates completed on these species across the studies and laboratories. The availability of data in both the SE and the VV tables for these species can be seen in Table 1 below. The “N” value for each sample indicates the number of replicates available for all studies. A replicate is defined as a pot, not as a single plant.

| <i>Species</i> | <i>CV</i> | <i>N</i> |
|----------------|-----------|----------|
| Corn | 1.71% | 144 |
| Soybean | 11.73% | 142 |
| Onion | 6.27% | 142 |
| Tomato | 9.54% | 134 |
| Cucumber | 5.95% | 122 |

Table 1A: SE 14-day emergence data by species.

| <i>Species</i> | <i>CV</i> | <i>N</i> |
|----------------|-----------|----------|
| Soybean | 51.0% | 975 |
| Onion | 37.0% | 957 |
| Corn | 47.0% | 948 |
| Tomato | 57.0% | 941 |
| Lettuce | 31.6% | 791 |

Table 1B: VV length data by species

| <i>Species</i> | <i>N</i> |
|----------------|----------|
| Onion | 614 |
| Soybean | 605 |
| Tomato | 584 |
| Corn | 568 |
| Cabbage | 500 |

Table 1C: VV weight data by species. The table indicates only “N” value because the C.V. was too incomparable across studies to add relevance.

Also important to note is the frequency of these four species being chosen by laboratories for testing. Table 2 shows the average CV for the five species tested in at least 8 of the 10

laboratories involved in the SE study. The CV indicates the variation across all studies in that laboratory on that specific crop.

Table 2: Average coefficient of variation of 14-day emergence data by species for each laboratory studied in SE tests; only those species studied by 8 or more laboratories were included.

| LabKey | Onion | Cucumber | Soybean | Tomato | Corn |
|--------|-------|----------|---------|--------|------|
| 1SE | 7.1% | 4.0% | 2.2% | 4.4% | 2.2% |
| 2SE | 5.8% | 4.0% | 6.1% | 5.0% | 3.5% |
| 4SE | 28.2% | 9.3% | 8.0% | 16.0% | 1.6% |
| 5SE | 7.2% | 5.1% | 3.9% | 5.6% | 1.2% |
| 6SE | 13.3% | 6.5% | 6.4% | 10.4% | 0.0% |
| 7SE | 15.6% | 12.9% | 7.2% | 38.8% | 0.0% |
| 8SE | 10.8% | 7.7% | 11.5% | 5.6% | 4.2% |
| 9SE | | 9.9% | 13.6% | | 7.7% |
| 10SE | 18.7% | 6.7% | 3.0% | 0.0% | 0.0% |

Seedling Emergence Studies

Across the laboratories conducting seedling emergence tests, most laboratories showed coefficients of variation (CV) less than 30% as can be seen on Figure 1 below. Soybean and corn exhibited the least variation, less than 20% for all laboratories, while tomato and onion plants showed greater but still a variation within the expected range of 20-30%, though tomatoes did produce an outlier of approximately 38% CV. The previously mentioned Table 2 points out the same pattern, as all computed CV values in the table are at or below 30%.

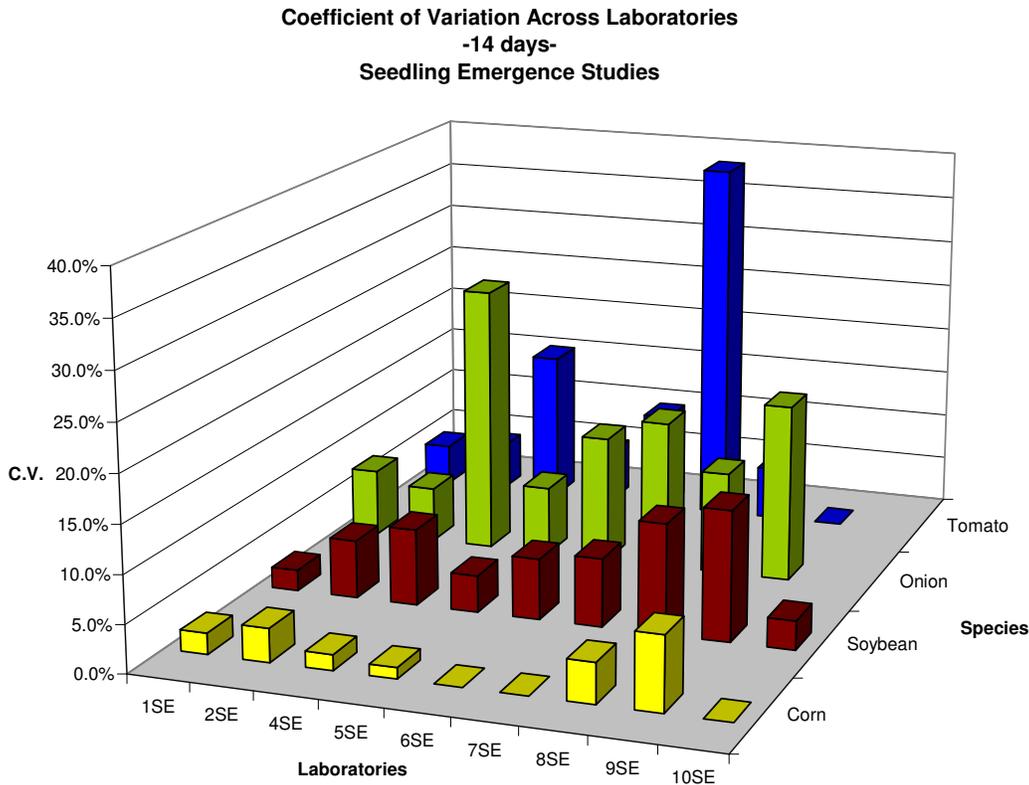


Figure 1: CV across laboratories for seedling emergence studies for frequently studied species.

To better understand intralaboratory variability, two laboratories were selected from the 10 surveyed based on the number of studies conducted and gathered from that laboratory. Labs 5SE and 6SE¹¹ conducted 12 and 8 studies, respectively, which were incorporated into the SE dataset. Therefore, these studies were chosen for further analysis to create the largest sample size possible within a laboratory.

In Figure 2 below, the interstudy variability for Lab 5SE is shown. The calculated CV values are all less than 20% (note that the left axis only rises to 18% CV). Corn and onion plants show the least amount of variation in emergence endpoints. Figure 3 below also shows little variation for corn plants with all studies indicating a CV of less than 5%. All the data for studies in these four species conducted at Lab 6SE peaks at approximately 28% CV.

¹¹ Please note that all laboratory names have been coded to abide by U.S. EPA confidentiality requirements.

**Coefficient of Variation within Laboratory 5SE
-14 days-
Seedling Emergence Studies**

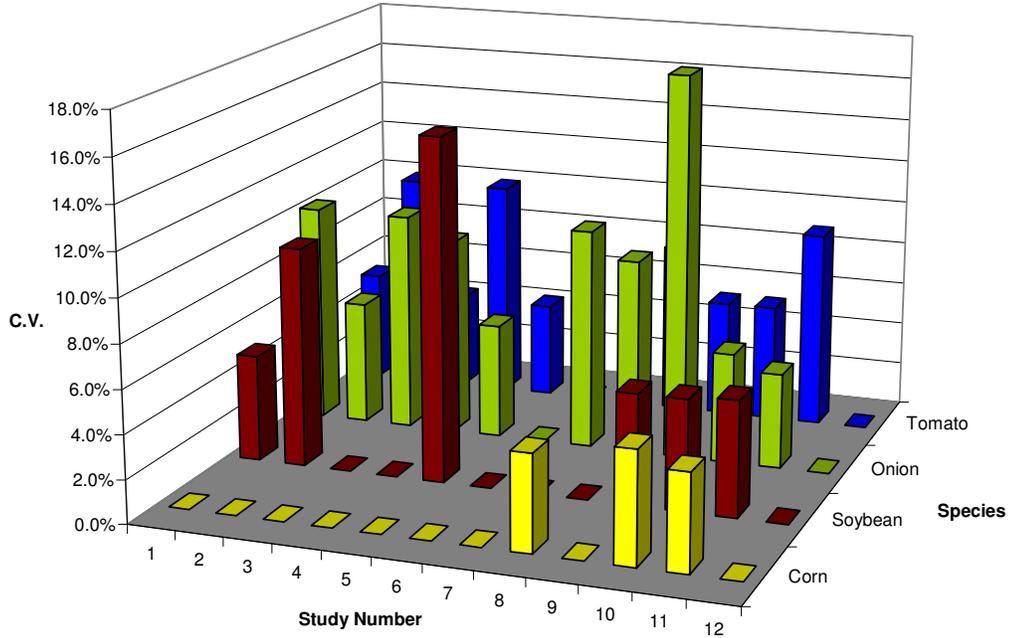


Figure 2: Coefficient of variation within laboratory 5SE for seedling emergence studies on selected species.

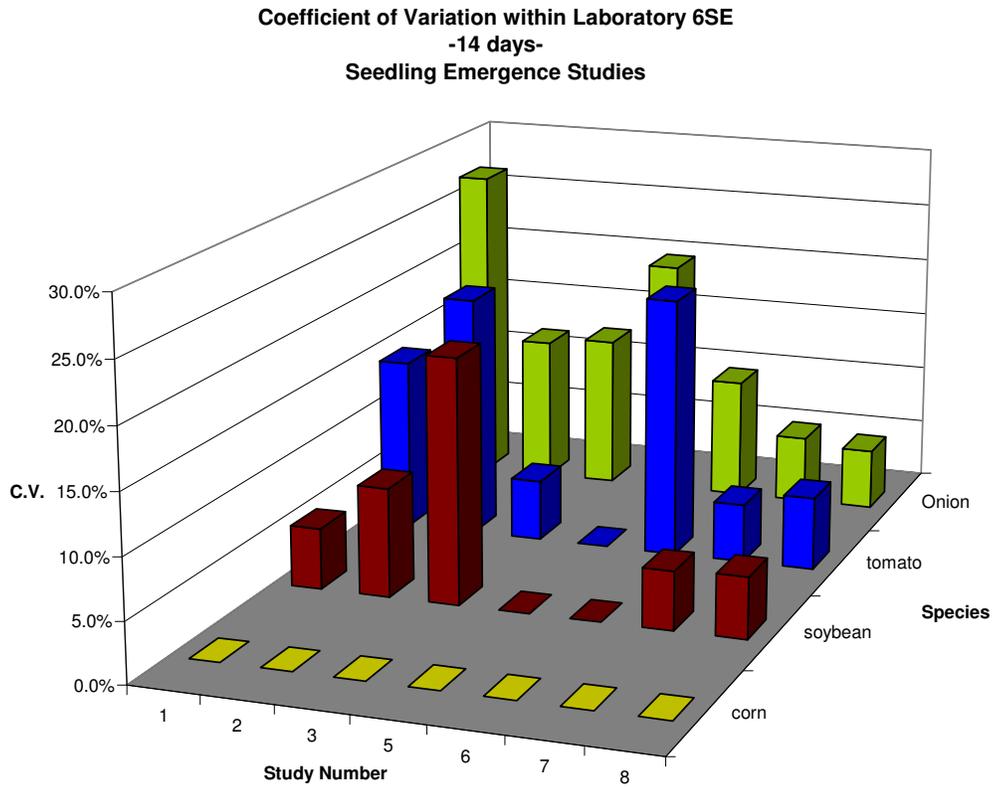


Figure 3: Coefficient of variation within laboratory 6SE for seedling emergence studies for frequently studied species.

The laboratory conditions and protocol recorded in each study have also been incorporated into the SE database and are summarized in the following paragraph. The quality of each study is broken down on a species level, meaning each species within a study has a classification code based on the adequacy of the conduct and achieved results for that endpoint. The classification is determined by the U.S. EPA. Each study, therefore, can have multiple classifications based on what occurred with each species. Of the captured seedling emergence studies, 48% (275 out of 571) were determined to be core, which indicates acceptable parameters and results. 18% of the studies were supplemental, indicating some issues but overall useful as additional data items, and 5% were invalid, indicating poor performance in the study.

Of the pots used, 40.5% were round (231 of 571) and 57.8% were square (330). The average surface area at the top of the pot was 369 cm², though values ranged from 56 - 1296 cm². The mode was 56 cm², as well. A majority of the studies used 1 pot per replicate, though the recorded range was 1-5 pots per replicate. The majority of pots (73.2%) held 10 plants, though values ranged from holding 2-20 plants per pot. The mean temperature was 21°C and the mean humidity was 43%.

Vegetative Vigor Studies

The variation across laboratories conducting VV studies was generally greater than that seen in the SE studies. Focusing on the plant weight endpoint, the variation in Lab 9VV exceeds that of the other laboratories studied, jumping to a CV of nearly 55% for corn. Excluding this study, the

CV is 40% or much less for all other labs and species. Again, a majority of the corn and soybean plants are below 30% CV and are generally much less variable than the tomato and onion plant replicates.

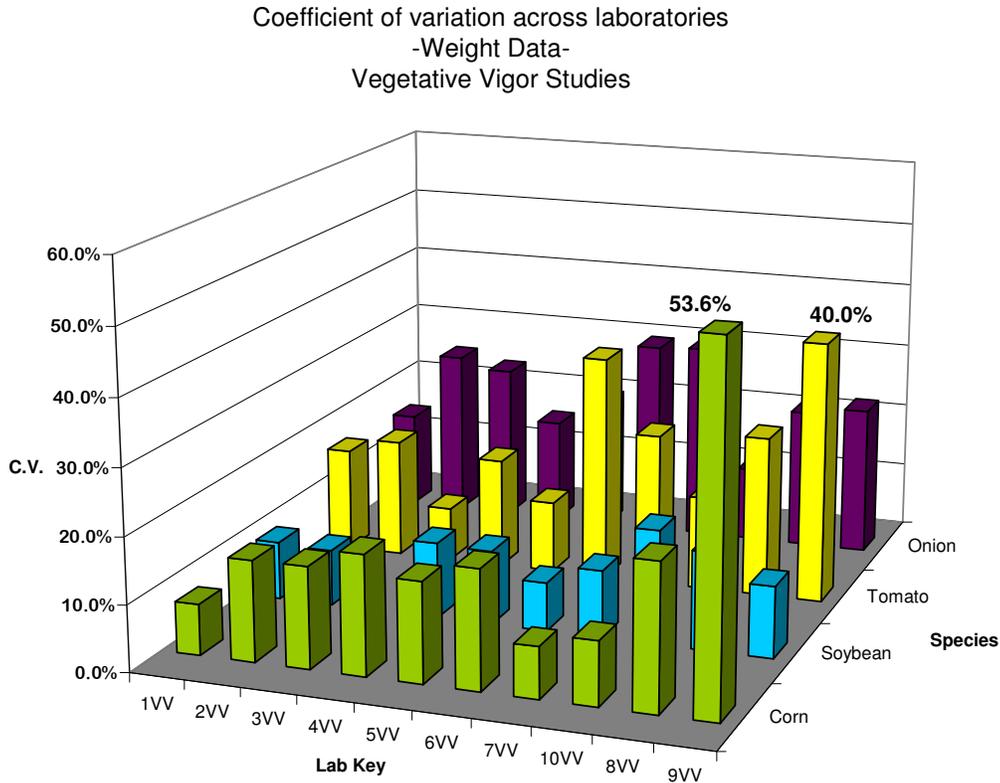


Figure 4: Coefficient of variation across laboratories for weight data from vegetative vigor studies for frequently studied species.

Patterns similar to the SE studies emerge for the VV length data endpoints as seen in Figure 5. All the CV values are less than 20% when considering the most frequently reported species. The most varied endpoint occurred in Lab 5VV in the onion plants, with a calculated CV of 14.1%. Also important to note is that Lab 5VV showed elevated variability in all species in comparison to the other laboratories.

Coefficient of variation across laboratories
 -Length Data-
 Vegetative Vigor Studies

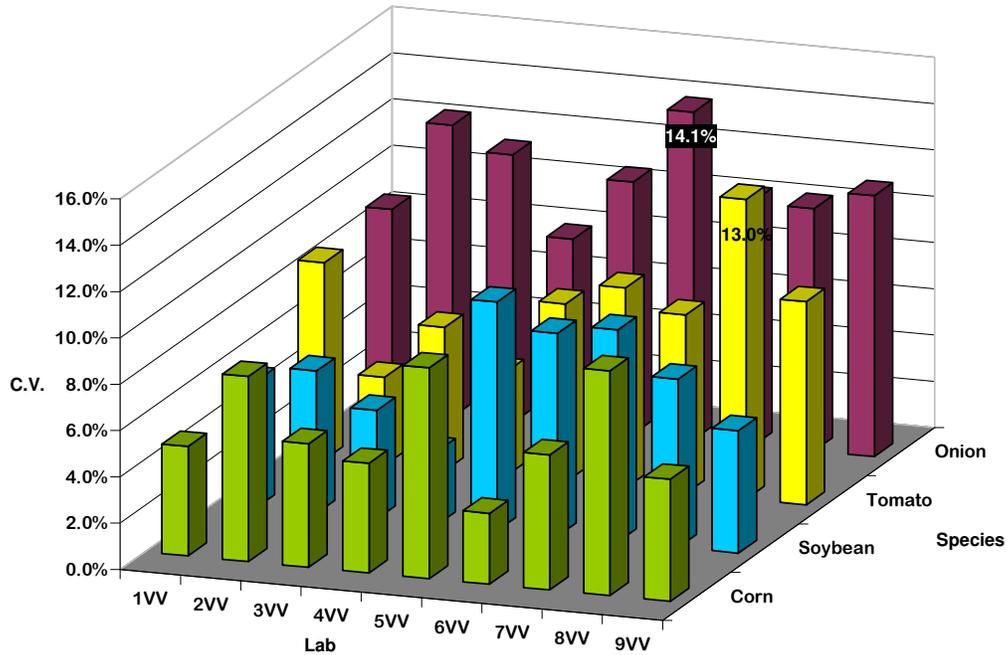


Figure 5: Coefficient of variation across laboratories for length data from vegetative vigor studies for frequently studied species.

Lab 1VV and Lab 8VV were selected for intralaboratory variability assessments as 10 studies including the target 4 species were captured for both labs. As seen in the weight data reported in Figures 6 and 7, substantially less variation is evident in the corn and soybean plants, much less than the expected 20%, in comparison to the onion and tomato plants which in some cases have a calculated CV of over 20%.

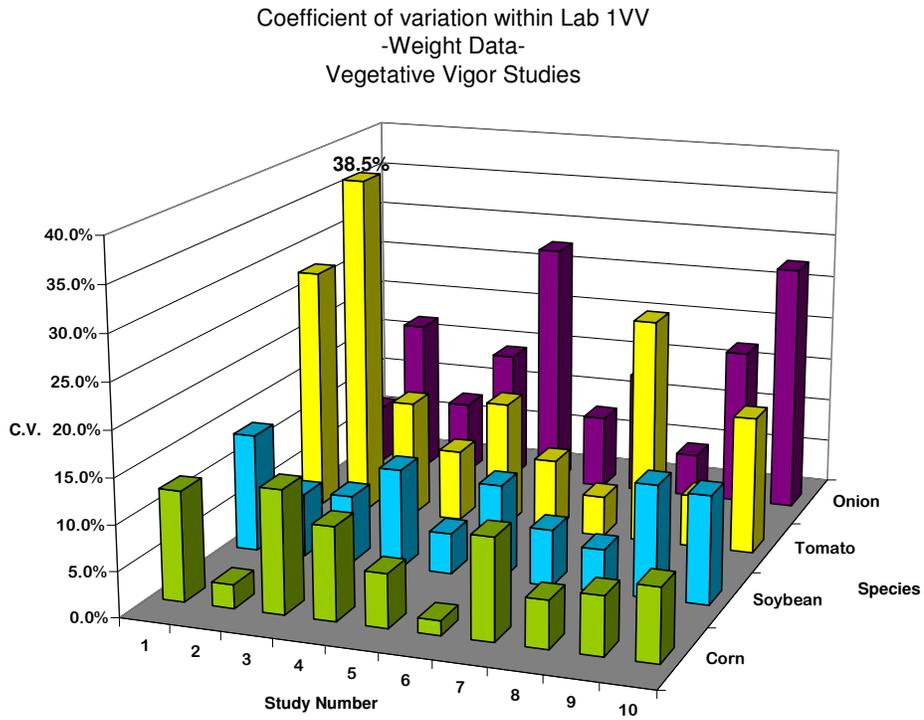


Figure 6: Coefficient of variation within laboratory 1VV for weight data collected from frequently studied species.

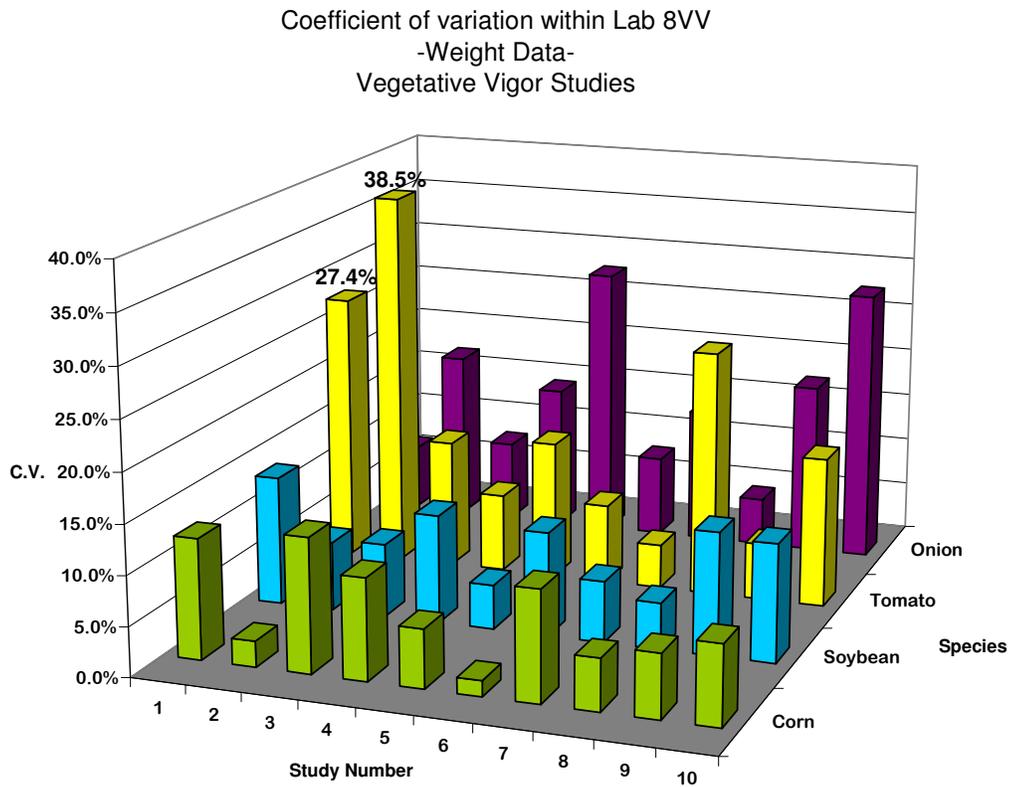


Figure 7: Coefficient of variation within lab 8VV for weight data on frequently studied species.

Figures 8 and 9 depict the variation in plant length from the VV studies. Lab 1VV shows variation below 15% with corn, soybean, and tomato plans, though onion is more varied yet does not reach 20%. For Lab 8VV (Figure 9), more variation is evident in the calculated CV values, however a majority of the studies remain below 30%.

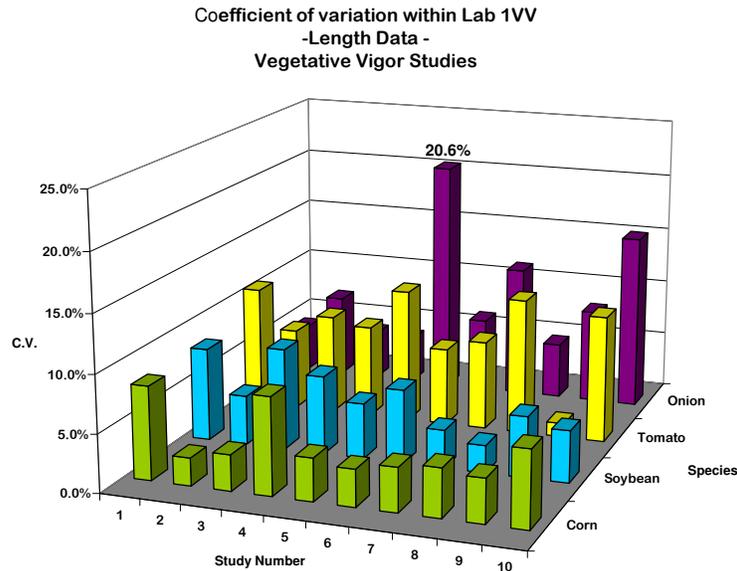


Figure 8: Coefficient of variation within lab 1VV for length data collected from frequently studied species.

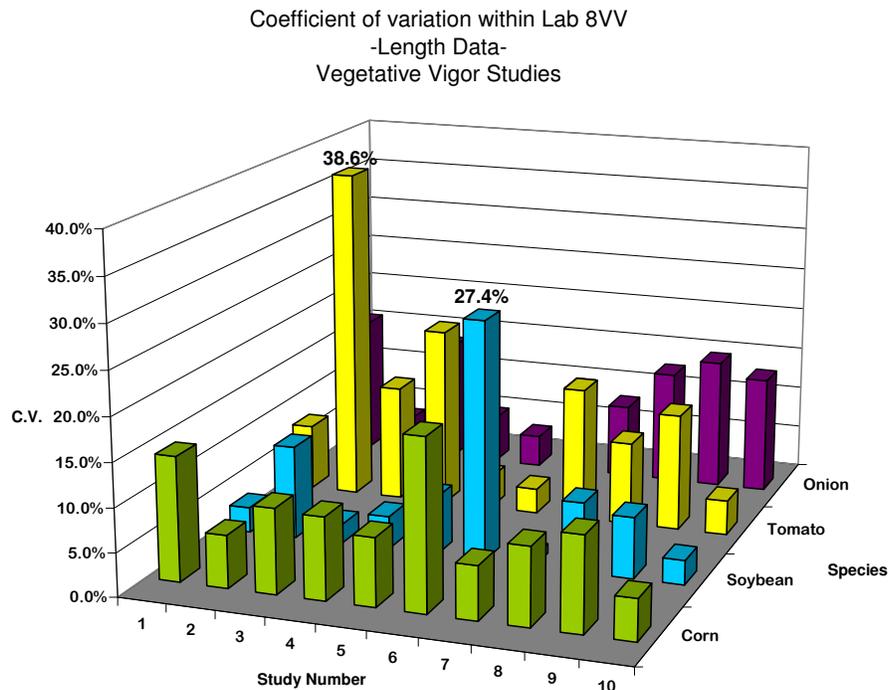


Figure 9: Coefficient of variation within lab 8VV for length data from frequently studied species.

The laboratory conditions recorded for the VV studies are similar to those observed in the SE studies. The lighting sources were relatively split, but most of the light was natural (26.5% of 69 studies) and the plants were subirrigated in some fashion (50%) with water from a well or municipal tap water supply (35%).

The average study duration was 17.6 days, though the median and the mode were 21. The average light intensity was 309 $\mu\text{mol}/\text{m}^2/\text{s}$ for an average of 14.3 hours. On average the temperature ranged from 19-31°C with a mean of 22°C.

Nearly 80% of the studies were classified as core, 15% were supplemental and only about 5% were invalid or unacceptable. A majority of the studies (60%) utilized square pots. 64.8% of the studies used one pot per replicate, but ranged from 1-8 pots per replicate, and in general five plants per pot were used, though the range was 1-50 plants per pot.

Discussion

The findings of the descriptive statistics revealed above support the currently accepted coefficient of variation (CV) of 20% used by the U.S. EPA. Both inter- and intra-laboratory variability shows calculated CV values of less than 40% with values generally residing below the 30% mark. For the SE database, all studies in all laboratories had average CV values of less than 40% and those laboratories responsible for conducting a majority of the studies (defined as 8 or more studies) exhibited even less variation with CV values less than 20%. Similar patterns were observed in the length data found in the VV database.

The weight data from the VV database, however, presented a different case wherein the variation was often greater than the expected 20-30% even for the most frequently tested species. Why this aberration arose is unclear, though the procedure for weighing plants is possibly more susceptible to error than measuring plant length and emergence. The weights are determined by drying all the plants in a replicate, so as to remove water weight, and weighing them as a unit, followed by dividing that unit by the number of plants in the replicate. If different amounts of water were left in the plants due to different drying conditions, it is possible for more variability to arise in the plant weights.

Looking at all of the interlaboratory graphs reveals a trend of higher variability across some labs rather than others. A similar pattern is seen in the lab-specific graphs which indicate study-level variation. These trends may imply a difference in the way a particular lab conducts its studies or the way a particular study is performed, rather than the variation of the plants. Therefore, it will be important for further analysis to uncover correlations between the laboratory conditions and protocol and variation seen at the laboratory and study level.

Among the species, the most data was available for corn and soybean since these two species are required given the 850 guidelines for terrestrial plant toxicity tests. The total number of measurements taken for these species exceeds that of all other species, though the values do not eclipse the total number of onion and tomato plants measured by too great a value. The CV is contingent upon the standard deviation, which is greatly tied to the N-value for a study. Therefore, the large N values of corn and soybean could be contributing to the low CV seen within and between the laboratories. Therefore, the corn and soybean plants may not be inherently less variable, instead seem less variable given the high number of replicates measured for the species. If the reason the CV appears below 10% for these species is based on the high availability of data, the inference is that all species may exhibit a similar, lower variation given enough data. Thus, if the U.S. EPA could acquire enough data to make sample sizes similar to that of the corn and soybean plants observed in these studies, the guidelines could ultimately be changed to reflect a more accurate CV of around 10%.

Study Continuation

Both of the VV and SE datasets can act as stand-alone data sources. However, since the target of 100 studies per dataset was never reached, both would benefit from additional data so as to generate more robust datasets. However, select laboratories conduct a majority of the studies for which the U.S. EPA and data from these laboratories have been incorporated into these datasets.

Adding studies from these select, or popular, laboratories will do nothing to better understand the interlaboratory variability of the tests. They studies could, however, prove potentially useful in improving the intralaboratory analysis, particularly for some of the species for which data is lacking, in other words all species but the ones that are generally chosen from the optional list, like tomato and onion. Additionally, data could be added regarding the phytotoxicity assessments from the studies already included. This was not included in the SE assessment. Seedling germination data could also be added, as that was excluded because germination studies are not a required part of the plant toxicity battery; and, therefore availability of data was limited.

Care should also be taken in future analyses to understand the implications of changing test guidelines and dates. These databases compiled all the possible control data, regardless of the time the test was conducted. Test guidelines, however, change overtime, like the OECD guidelines which shifted in 2006 from standards set in 1984.¹² The U.S. EPA similarly set guidelines in 1986 which were slightly amended in 2007.¹³ Changes to guidelines usually include more limitations on the possible test species or environmental conditions. Interestingly, both factors contributed to the variation observed in the studies. Therefore, analyzing this data with a mind towards the year the test was completed might yield a more prudent assessment.

Further analyzing environmental conditions and other protocol variations would be useful. These likely affect variability of plant growth. The first examination might focus on those labs where the variability has spiked, in comparison to other labs, and might include temporal comparisons within that lab.

¹² Organization for Economic Cooperation and Development. 2007. OECD Guidelines for Testing of Chemicals. <http://www.oecd.org/dataoecd/9/11/33663321.pdf>

¹³ U.S. Environmental Protection Agency, 1986. Hazard Evaluation Division, Standard Evaluation Procedure - Nontarget Plants: Seed Germination/Seedling Emergence -Tiers 1 and 2. EPA 540/9-86-132.

Conclusion

The historical value of a 20% coefficient of variation seems to be, on average, an acceptable value for the terrestrial plant toxicity tests the US EPA reviews for pesticide registration. The data of this report indicates particular species, specifically corn and soybean, tend to have lower CV, approximating values closer to 10%. The addition of more data and a more sophisticated analysis into the underlying causes of the variation which is evident in this initial data collection could result in a lesser CV across all species. Additionally, the U.S. EPA could consider an additional assessment of the guidelines. For example, setting a different standard for these such as is done for the “keystone” species of other ecological risk assessments when the sensitivity of a particular species is known, thus establishing more of a tiered analytical system rather than a “one-size fits all” approach to terrestrial plant growth variations.

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Appendix 2: Additional Abstract

Abstract
Evaluating Variation in Terrestrial Plant Toxicity Tests
by
Lindsey Kurnath
May 2008

The Office of Pesticide Programs of the U.S. EPA conducts the national pesticide review process, under authority granted by FIFRA. Ecological risk assessments are performed on many different organisms to identify ecosystem-level impacts. Among these organisms are non-target terrestrial plants, which are specifically used to understand the risk to plants exposed to spray drift or runoff. Due to the development of guidelines from field-based methods rather than a traditional interlaboratory precision analysis, the natural variation in measurement endpoints for terrestrial plant toxicity tests has not been established. With a better grasp of natural variation, the EPA could require more precise data for pesticide assessments on plants. Recognizing the inherent sensitivity of the assessments they are currently conducting is important for the U.S. EPA to be able to better understand the implications of the pesticide review process.

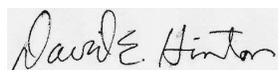
The following report aims to determine the natural variability of the response endpoints (i.e. emergence, plant length, plant weight) for these studies. This project involved the development and design of two databases in MS Access, the selection of control data from existing vegetative vigor and seedling emergence studies, an analysis of the among and within laboratory variability in response measures, and a trend analysis for variation due to environmental conditions.

The findings of the descriptive statistics supported the currently accepted coefficient of variation (CV) of 20% used by the U.S. EPA. All seedling emergence studies in all laboratories had average CV values of less than 40% and laboratories who were responsible for conducting a majority of the studies exhibited CV values less than 20%. Similar patterns were observed in the length data from the vegetative vigor studies. The weight data presented variation that was often greater than the expected 20 even for the most frequently tested species. The most data was available for corn and soybean plants and these species were also the least variable.

The historical value of a 20% coefficient of valuation seems to be, on average, an acceptable value for the terrestrial plant toxicity tests used for pesticide registration. The data of this report indicates particular species, specifically corn and soybean, tend to have lower CV values closer to 10%. The addition of more data and a more sophisticated analysis into the underlying causes of variation could reveal a lower CV across all species.

Date: 4/23/08

Approved:



Dr. David Hinton, Advisor

Masters project submitted in partial fulfillment of the
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Environment and Earth Sciences of Duke University 2008

Appendix 3: Supplemental Data Tables

Table 2: Full list of Species included in both seedling emergence and vegetative vigor databases.

| Species | Study | |
|--------------------------------|-------|----|
| | SE | VV |
| <i>Abutilon theophrasti</i> | SE | |
| <i>Allium cepa</i> | SE | VV |
| <i>Avena fatua</i> | SE | VV |
| <i>Avena sativa</i> | SE | VV |
| <i>Beta vulgaris</i> | SE | VV |
| <i>Brassica campestris</i> | SE | VV |
| <i>Brassica kaber</i> | SE | |
| <i>Brassica napus</i> | SE | VV |
| <i>Brassica oleracea</i> | SE | VV |
| <i>Brassica rapa</i> | SE | VV |
| <i>Cucumis sativus</i> | SE | VV |
| <i>Cyperus rotundus</i> | SE | |
| <i>Daucus carota</i> | SE | VV |
| <i>Echinochloa crus-galli</i> | SE | VV |
| <i>Fagopyrum esculentum</i> | SE | |
| <i>Fagopyrum sagittatum</i> | SE | VV |
| <i>Glycine max</i> | SE | VV |
| <i>Gossypium hirsutum</i> | | |
| <i>Helianthus annuus</i> | SE | VV |
| <i>Ipomoea hederacea</i> | SE | |
| <i>Lactuca sativa</i> | SE | VV |
| <i>Linum usitatissimum</i> | SE | |
| <i>Lolium perenne</i> | SE | VV |
| <i>Lycopersicon esculentum</i> | SE | VV |
| <i>Phaseolus vulgaris</i> | SE | VV |
| <i>Pisum sativum</i> | SE | VV |
| <i>Raphanus sativus</i> | SE | VV |
| <i>Sorghum bicolor</i> | SE | VV |
| <i>Sorghum vulgare</i> | SE | VV |
| <i>Triticum aestivum</i> | SE | VV |
| <i>Xanthium strumarium</i> | SE | |
| <i>Zea mays</i> | SE | VV |

Appendix 4: Data Entry Protocol Sample – Vegetative Vigor Database

Rationale

The within and between laboratory variability for endpoints in the vegetative vigor study, for any plant species, has not been evaluated. The 850 vegetative vigor guidelines, version 2006, include a statement that the coefficient of variation (CV) of controls should be 20% or less, though that number has not been verified experimentally for any species. As it is not possible to control the inherent variability, there is a need to establish the expected within and between laboratory variability, for at least guideline recommended species, and evaluate if the guideline statement about the CV is practical and relevant for the species tested.

As it is not practical, due to resource limitations and timeframe constraints, to conduct ring tests to measure within and between laboratory variability another approach is to analyze the within and between laboratory variability using control data from existing tests. The analysis process includes the development of a database of vegetative vigor studies containing biomass measures (i.e., length, weight) for the controls and study design and environmental factors that affect variability (e.g., light source, light intensity, pot size) and a statistical analysis to estimate within and between laboratory variability with considerations of covariates. The following document outlines the database constructed to meet the needs of the analysis.

General Guide

The Vegetative Vigor Database (VVDB) was built using Microsoft Access, in which tables were linked to minimize the data entry process while maximizing data integrity. Some tables were created with the inclusion of a “primary key”, which is a unique data point identifying the entry. When designated a primary key, the field cannot contain another entry with duplicate information. A “foreign key” can be duplicated in the same table as well as linked to a primary key designated in another table.

The variable definitions in the following pages describe what information went into each field as well as how that information should be entered. Where the fields require the data be classified (i.e. Light Source, Water Source, Classification), the options have been listed and explained where necessary.

Where data information was not provided in the study, the cells were left blank or have zeros. In the cases where more than one value was listed in the study, the data was entered in a supplemental subform. The table organization is described below. An accompanying diagram follows the text as well as a picture of the form used for data entry.

Table Identification and Variable Definitions

The Study Info Table contains data applicable to the entire study as indicated below. The primary key for the table is the MRID number. Therefore, each study has one Study Info Table, designated by the MRID number.

Table 3: Study Info Table Variables

| <i>Field Name</i> | <i>Definition</i> | <i>Type</i> | <i>Format</i> |
|-------------------|-------------------|-------------|---------------|
| MRID | EPA ID for study | Text | 8 Characters |

| | | | |
|-------------------------|--|--------|----------------|
| Chemical name | Name of chemical being tested | Text | 50 Characters |
| LabID | Three letter abbreviation for laboratory conducting study | Text | 3 Characters |
| Study Start Date | Start date of study | Date | MM/DD/YYYY |
| Study Duration | Length of vegetative vigor growth observations study | Number | Integer |
| DUR Units | Study duration units | Text | 50 Characters |
| Light Source | Light source used in study, classified as one of the following: Natural; Incandescent; Fluorescent; Both I&F; Nat & Incan; Nat & Fluor; Nat & Other | Text | Fixed value |
| Watts (I) | Wattage of incandescent light source | Number | Integer |
| Watts (F) | Wattage of fluorescent light source | Number | Integer |
| Other light source info | Additional pertinent light source info | Text | 255 Characters |
| Min LI | Minimum light intensity during study | Number | Decimal |
| Max LI | Maximum light intensity during study | Number | Decimal |
| Mean LI | Mean light intensity during study | Number | Decimal |
| Var LI | Variance (e.g., SD, SE, etc.) of light intensity during study | Number | Decimal |
| LI Units | Units of light intensity measurement | Text | 50 Characters |
| LI Duration | Daily duration of plant exposure to light source | Number | Integer |
| LI D Units | Light intensity duration measurement units | Text | 50 Characters |
| Water Method | Watering method used in the study, classified as one of the following: Bottom, Under Canopy, Overhead | Text | Fixed value |
| Water Source | Source of the water used for the study | Text | 50 Characters |
| Min Temp | Minimum temperature reached during study | Number | Decimal |
| Mean Temp | Mean temperature reached during study | Number | Decimal |
| Max Temp | Maximum temperature reached during study | Number | Decimal |
| Temp Units | Units of temperature measurement | Text | 50 Characters |
| Temp Notes | Additional information on temperature measurements | Text | 255 Characters |
| Min Humidity | Minimum relative humidity reached during study expressed as a percentage | Number | Decimal |
| Mean Humidity | Mean relative humidity reached during study expressed as a percentage | Number | Decimal |
| Max Humidity | Maximum relative humidity reached during study expressed as a percentage | Number | Decimal |

| | | | |
|----------------|--|------|----------------|
| Humidity Notes | Additional information on relative humidity measurements | Text | 255 Characters |
|----------------|--|------|----------------|

The Laboratory Name Table includes necessary information regarding the laboratory conducting the study and abbreviations used in data entry. The LabID is the link between this table and the Study Info Table, and the trigger to autopopulate the Laboratory Name field on the Study Info form with the Lab Name from the Laboratory Name table. The primary key for this table is the LabID. All laboratory names and LabID designations must be entered via the original table, prior to data entry on the form, to maintain a list of laboratory names which can be 100% quality assured. Each laboratory should have a unique LabID and neither field should be repeated elsewhere in the table.

Table 4: Laboratory Name Table Variables

| <i>Field Name</i> | <i>Definition</i> | <i>Type</i> | <i>Format</i> |
|-------------------|---|-------------|---------------|
| LabID | Three letter EPA abbreviation for laboratory conducting study | Text | 3 Characters |
| Full Name | Full name of laboratory | Text | 50 Characters |

The Chemical Info Table contains information regarding the chemical being tested and its associated identification numbers. The Chemical Name is the link between this table and the Study Info Table, and is the trigger to autopopulate the CAS# and PC Code on the Study Info Form with the corresponding fields from the Chemical Info Table. The primary key for this table is the Chemical Name. All chemical names, CAS#, and PC code designations must be entered via the original table, prior to data entry on the form, to maintain a list of chemical names which can be 100% quality assured. The source for the Chemical Info Table is the National Pesticide Information Retrieval System from Purdue University, <http://ppis.ceris.purdue.edu/htbin/cnamkeys.com>.

Table 5: Chemical Info Table Variables

| <i>Variable</i> | <i>Definition</i> | <i>Type</i> | <i>Format</i> |
|-----------------|---|-------------|---------------|
| Chemical Name | Name of chemical being tested | Text | 50 Characters |
| CAS# | Chemical Abstracts Service registry number for test chemical | Number | 50 Characters |
| PC Code | EFEDs active ingredient reference number (Shaughnessy number) | Number | 50 Characters |

The Photoperiod Table holds detailed light exposure information on the study when more than one value for photoperiod or light intensity duration was included in the study. This table is linked to the Study Info Table via the MRID number and is only filled in when the additional information is supplied in the raw data.

Table 6: Photoperiod Table Variables

| <i>Variable</i> | <i>Definition</i> | <i>Type</i> | <i>Format</i> |
|-----------------|--------------------------------|-------------|---------------|
| MRID | EPA identifier for study | Text | 8 Characters |
| Date | Date the measurement was taken | Date | MM/DD/YYYY |

| | | | |
|-------------------|--|--------|----------------|
| Photoperiod | Length of time plants were exposed to light source | Number | Decimal |
| Photoperiod Units | Units of photoperiod measurement | Text | 50 Characters |
| Start | Start time of photoperiod (e.g. sunrise) | Time | HH:MM A or P |
| End | End time of photoperiod (e.g. sunset) | Time | HH:MM A or P |
| Notes | Applicable notes on photoperiod or light data | Text | 255 Characters |

The Light Intensity Table includes records pertinent to the study-specific light intensity data when more than one value for light intensity parameters is included in the study. The MRID number links this table to the Study Info Table. Like the Photoperiod Table, the Light Intensity Table was only filled in the case of raw data or multiple data points being available.

Table 7: Light Intensity Table Variables

| <i>Variable</i> | <i>Definition</i> | <i>Type</i> | <i>Format</i> |
|-----------------|--|-------------|----------------|
| MRID | EPA identifier for study | Text | 8 Characters |
| Date | Date the measurement was recorded | Date | MM/DD/YYYY |
| Time | Time the measurement was recorded | Time | HH:MM A or P |
| Date Range | Date range over which the measurements were observed | Text | 50 Characters |
| Mean LI | Mean light intensity recorded for study period | Number | Decimal |
| Min LI | Minimum light intensity recorded for study period | Number | Decimal |
| Max LI | Maximum light intensity recorded for study period | Number | Decimal |
| LI Range | Light intensity range reported for the study period | Text | 50 Characters |
| LI Units | Units of light intensity measurement | Text | 50 Characters |
| Notes | Applicable notes on light intensity data | Text | 255 Characters |

The Environmental Conditions Table contains data applicable to temperature and relative humidity measurements recorded during the study. This table is linked to the Study Info Table through the MRID number. Detailed environmental data was recorded only when raw data or multiple data points were available in the original study.

Table 8: Environmental Conditions Table Variables

| <i>Variable</i> | <i>Definition</i> | <i>Type</i> | <i>Format</i> |
|-----------------|---|-------------|---------------|
| MRID | EPA identifier for study | Text | 8 Characters |
| Date | Date the measurement was recorded | Date | MM/DD/YYYY |
| Time | Time the measurement was recorded | Time | HH:MM A or P |
| Greenhouse | Location within greenhouse measurement was take (e.g. greenhouse descriptor, front or rear, etc.) | Text | 50 Characters |
| Min Temp | Minimum temperature reached during study | Number | Decimal |
| Mean Temp | Mean temperature reached during study | Number | Decimal |
| Max Temp | Maximum temperature reached during study | Number | Decimal |
| SD Temp | Standard deviation of temperature measurements | Number | Decimal |
| Min Humidity | Minimum relative humidity reached during study expressed | Number | Decimal |

| | | | |
|---------------|--|--------|---------|
| | as a percentage | | |
| Mean Humidity | Mean relative humidity reached during study expressed as a percentage | Number | Decimal |
| Max Humidity | Maximum relative humidity reached during study expressed as a percentage | Number | Decimal |
| SD Humidity | Standard deviation of relative humidity measurements | Number | Decimal |

The Species Name Table has identifying information for the test species. It is linked to the Design Info Table through the Scientific Name variable. Entering a name in the Scientific Name field prompts the Common Name, Family, Class, and Root Crop names to autopopulate in the Design Info Form off of the data supplied in the Species Name Table. The primary key is the Scientific Name. All species names and common names must be entered via the original table, prior to data entry on the form, to maintain a list of laboratory names which can be 100% quality assured. The source for all the contents of the Species Name Table, minus the Root Crop classification, can be found on the USDA Plant Database website at <http://plants.usda.gov/java/nameSearch>. Root Crop classification came following a consult from a plant biologist in EFED, and this category should be quality controlled similarly.

Table 9: Species Name Table Variables

| <i>Variable</i> | <i>Definition</i> | <i>Type</i> | <i>Format</i> |
|-----------------|--|-------------|---------------|
| Scientific Name | Generally accepted scientific name of test species | Text | 50 Characters |
| Common Name | Common name of test species | Text | 50 Characters |
| Family | Family classification of test species | Text | 50 Characters |
| Class | Cotyledon class: Monocot or Dicot | Text | Fixed Value |
| Root Crop | Root crop classification: Yes, No, or Maybe (depends on usage) | Text | Fixed Value |

The Design Info Table pertains to study-specific physical and temporal parameters of the study. It is linked to the Study Info Table through the MRID number. The primary key is a multiple-field key, combining the MRID and the Scientific Name. For each species studied in the experiment, the scientific name is appropriately entered and a unique MRID-Scientific Name combination is generated. Therefore, one study may have multiple Design Info Table entries for one entry in the Study Info Table.

To include as much information as possible per replicate on planting conditions, pot characterization was broken down in to multiple components. If the pot shape was reported as square, the Pot Length and Pot Width fields were filled in as the pot dimensions. If the pot shape was reported as round, the Pot Diameter (Top) and the Pot Diameter (Bottom) were filled in as the pot dimensions.

Table 10: Design Info Table Variables

| <i>Variable</i> | <i>Definition</i> | <i>Type</i> | <i>Format</i> |
|-----------------|------------------------------------|-------------|---------------|
| MRID | EPA ID for study | Text | 8 Characters |
| Scientific Name | Generally accepted scientific name | Text | 50 Characters |

| | | | |
|-----------------------|---|--------|---------------|
| | of test species | | |
| Classification | Classification as determined by EFED: Core, Supplemental, Invalid, Acceptable, Unacceptable | Text | Fixed value |
| Pot Volume | Reported volume of the planting pot used in the study | Number | Decimal |
| Pot Shape | Shape of the pot: round or square | Text | Fixed Value |
| Pot Height | Height of planting pot used for replicates | Number | Decimal |
| Pot Diameter (Top) | Top diameter of round pot used for replicates | Number | Decimal |
| Pot Diameter (Bottom) | Bottom diameter of round pot used for replicates | Number | Decimal |
| Pot Length | Length of square pot used for replicates | Number | Decimal |
| Pot Width | Width of square pot used for replicates | Number | Decimal |
| Pot Units | Units of pot size measurement | Text | 50 Characters |
| Pots per rep | Number of pots per replicate | Number | Fixed integer |
| Plants per pot | Number of individual plants per pot | Number | Fixed integer |

The Weight Data Table is linked to the Study Design Table using the unique ID combination of the MRID and the Scientific Name. This table addresses the recorded weights per replicate measured in the study. If only one weight is recorded per replicate, the weight value indicates the mean weight of the replicate. If more than one weight value is recorded per replicate, the weight is indicative of an individual plant length measurement.

Table 11: Weight Data Table Variables

| <i>Variable</i> | <i>Definition</i> | <i>Type</i> | <i>Format</i> |
|-----------------|--|-------------|---------------|
| MRID | EPA ID for study | Text | 8 Characters |
| Scientific Name | Generally accepted scientific name of test species | Text | 50 Characters |
| Rep | Replicate number ID | Text | Fixed integer |
| Weight | Measured weight of aboveground biomass of plant | Number | Decimal |
| W Units | Units of weight measurement | Text | 50 Characters |

The final data table is the Length Data Table, which includes the information regarding experimentally recorded replicates for plant length. It is linked to the Study Design Table using the same unique ID combination of the MRID and the Scientific Name. If only one length is recorded per replicate, the length value indicates the mean length of the replicate. If more than one length is recorded per replicate, the length is indicative of an individual plant weight measurement.

Table 12: Length Data Table Variables

| <i>Variable</i> | <i>Definition</i> | <i>Type</i> | <i>Format</i> |
|-----------------|---|-------------|---------------|
| MRID | EPA unique ID for study | Text | 8 Characters |
| Scientific Name | Scientific name of test species | Text | 50 Characters |
| Rep | Replicate number ID | Text | Fixed integer |
| Length | Measured length of aboveground biomass of plant | Number | Decimal |
| L Units | Units of plant length measurement | Text | 50 Characters |

Upon the completion of data entry in the base set of tables, two summary tables were created to consolidate the dataset. The Study Info_Summary Table is composed of information from the Study Info, Photoperiod, Light Intensity, and Enviro Conditions Tables. Summary statistics were run on the data from the latter three tables to create fields consistent with those contained in the Study Info table. By creating a new table that reads off the Study Info Table but allows for the insertion of summary data statistics from the latter three tables, the integrity of the original dataset is preserved. Like the source data table, the Study Info_Summary table includes a primary key set as the MRID number so each entry has a specific identifier. It is also important to note all the data has been converted to standardized units appropriate for each field, which allow for better comparison between values.

Table 13: Study Info_Summary Table Variables

| <i>Field Name</i> | <i>Definition</i> | <i>Type</i> | <i>Format</i> |
|-------------------|--|-------------|---------------|
| MRID | EPA ID for study | Text | 8 Characters |
| Chemical name | Name of chemical being tested | Text | 50 Characters |
| LabID | Three letter abbreviation for laboratory conducting study | Text | 3 Characters |
| Study Start Date | Start date of study | Date | MM/DD/YYYY |
| Study Duration | Length of vegetative vigor growth observations study | Number | Integer |
| DUR Units | Study duration units | Text | 50 Characters |
| Light Source | Light source used in study, classified as one of the following: Natural; Incandescent; Fluorescent; Both I&F; Nat & Incan; Nat & Fluor; Nat & Other | Text | Fixed value |
| Watts (I) | Wattage of incandescent light source | Number | Integer |
| Watts (F) | Wattage of fluorescent light source | Number | Integer |
| Min LI | Minimum light intensity during study | Number | Decimal |
| Max LI | Maximum light intensity during study | Number | Decimal |
| Mean LI | Mean light intensity during study | Number | Decimal |
| Var LI | Variance (e.g., SD, SE, etc.) of light intensity during study | Number | Decimal |
| LI Units | Units of light intensity measurement | Text | 50 Characters |
| LI Duration | Daily duration of plant exposure to | Number | Integer |

| | | | |
|---------------|---|--------|---------------|
| | light source | | |
| LI D Units | Light intensity duration measurement units | Text | 50 Characters |
| Water Method | Watering method used in the study, classified as one of the following: Bottom, Under Canopy, Overhead | Text | Fixed value |
| Water Source | Source of the water used for the study | Text | 50 Characters |
| Min Temp | Minimum temperature reached during study | Number | Decimal |
| Mean Temp | Mean temperature reached during study | Number | Decimal |
| Max Temp | Maximum temperature reached during study | Number | Decimal |
| Var Temp | Standard deviation of temperature during study | Number | Decimal |
| Temp Units | Units of temperature measurement | Text | 50 Characters |
| Min Humidity | Minimum relative humidity reached during study expressed as a percentage | Number | Decimal |
| Mean Humidity | Mean relative humidity reached during study expressed as a percentage | Number | Decimal |
| Max Humidity | Maximum relative humidity reached during study expressed as a percentage | Number | Decimal |
| Var Humidity | Standard deviation of relative humidity during study | Number | Decimal |

The Design Info Table is also reiterated on the Design Info_Summary Info Table with conversions and calculations to create a more uniform database for analysis. Like the Study Info_Summary Table, the Design Info_Summary Table allows for a uniform database while maintaining the integrity of the original dataset.

Table 14: Design Info_Summary Table Variables

| <i>Variable</i> | <i>Definition</i> | <i>Type</i> | <i>Format</i> |
|------------------|---|-------------|---------------|
| MRID | EPA ID for study | Text | 8 Characters |
| Scientific Name | Generally accepted scientific name of test species | Text | 50 Characters |
| Classification | Classification as determined by EFED: Core, Supplemental, Invalid, Acceptable, Unacceptable | Text | Fixed value |
| Pot Surface Area | Calculated surface area at the top of the planting pot | Number | Decimal |
| Pot Shape | Shape of the pot: round or square | Text | Fixed Value |
| Pot units | Units of pot size measurement | Text | 50 Characters |
| Pots per rep | Number of pots per replicate | Number | Fixed integer |

| | | | |
|----------------|-------------------------------------|--------|---------------|
| Plants per pot | Number of individual plants per pot | Number | Fixed integer |
|----------------|-------------------------------------|--------|---------------|

The linkages between all the tables are illustrated in Figure 1 and the data entry form, including labeling of each section corresponding to the previously described tables, is show in Figure 2.

Figure 10: Relationships between tables as seen in MS Access, including table and variable names

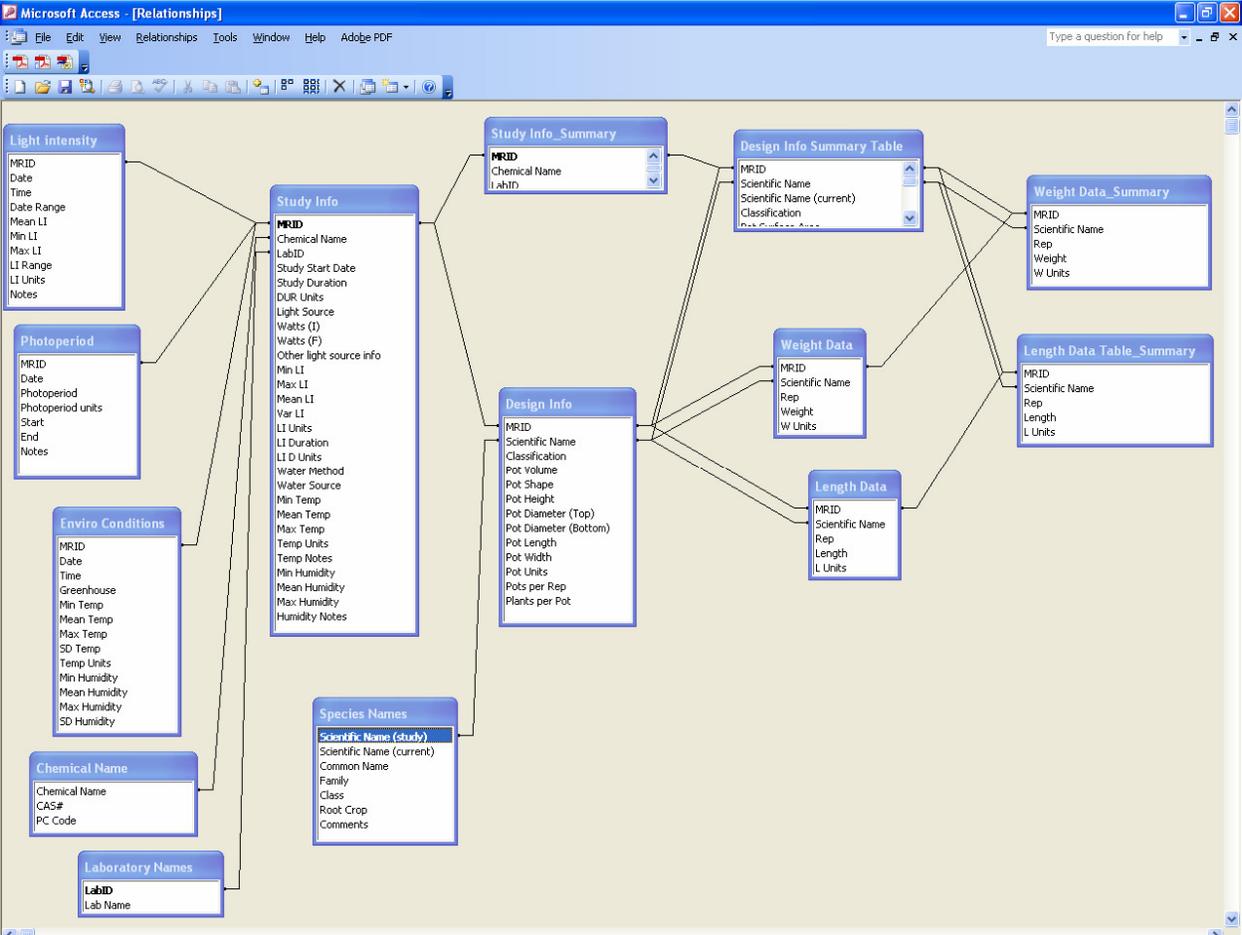


Figure 11: Data entry form as it appears in MS Access. The tables are indicated as follows: 1. Study Info, 2. Photoperiod, 3. Light Intensity, 4. Environmental Conditions, 5. Design Info, 6. Length Data, 7. Weight Data.

MRID: Study Start: 10/23/1987 Light Sour: Natural Water Mef: Overhead
 Chemical I: Study Dur: 21 Watts (I): 0 Water Sox: Open Summary Form
 CAS#: DUR Units: days Watts (F): 0
 PC Code: Min LI: 0 Other light: Min Temp: 0 Min Humic:
 LabID: Max LI: 0 LI Duration: 0 Mean Ten: 0 Mean Hum:
 Lab Name: Near: 0 LI Units: Max Temp: 0 Max Humi:
 Var LI: LI D Units: Temp Unit: Humidity N:
 Temp No: no T or H data given, only charts

Photoperiod

| MRID | Date | Photoperiod | Photoperiod units | Start | End | Notes |
|------|------------|-------------|-------------------|---------|---------|-------|
| | 10/23/1987 | 0 | | 7:05 AM | 6:10 PM | |
| | 10/24/1987 | 0 | | 7:06 AM | 6:09 PM | |
| | 10/25/1987 | 0 | | 6:07 AM | 5:08 PM | |
| | 10/26/1987 | 0 | | 6:07 AM | 5:06 PM | |

Records: 14 of 27

Light intensity

| MRID | Date | Time | Date Range | Mean LI | Min LI | Max LI | LI Range | LI Units | Notes |
|------|------------|---------|------------|---------|--------|--------|----------|----------|-------|
| | 10/23/1987 | 9:00 AM | | 1500 | 0 | 0 | | ftc | |
| | 10/26/1987 | 9:00 AM | | 1300 | 0 | 0 | | ftc | |
| | 10/27/1987 | 9:00 AM | | 1300 | 0 | 0 | | ftc | |
| | 10/23/1987 | 9:00 AM | | 1000 | 0 | 0 | | ftc | |

Records: 14 of 54

Enviro Conditions

| MRID | Date | Time | Greenhouse | Min Temp | Mean Temp | Max Temp | SD Temp | Temp Units | Min Humidity | Mean Humidity | Max Humidity |
|----------|------|------|------------|----------|-----------|----------|---------|------------|--------------|---------------|--------------|
| 40728323 | | | | 0 | 0 | 0 | 0 | 0 | | | |

Records: 14 of 1

Design Info

MRID: Classification: Core
 Scientific Name: Glycine max Pot Volume: 0 Pot Diameter (Bottom): 0
 Common Name: Soybean Pot Shape: square Pot Length: 7.5
 Family: Fabaceae Pot Height: 6 Pot Width: 7.5
 Class: Dicot Pot Diameter (Top): 0 Pot Units: cm
 Root Crop: No Pots per Rep: 1
 Comments: Plants per Pot: 5

Length Data

| MRID | Scientific Name | Rep | Length | L Units |
|------|-----------------|-----|--------|---------|
| | Glycine max | 1 | 209 mm | |
| | Glycine max | 1 | 259 mm | |
| | Glycine max | 2 | 247 mm | |
| | Glycine max | 1 | 238 mm | |
| | Glycine max | 1 | 239 mm | |
| | Glycine max | 2 | 269 mm | |

Records: 14 of 15

Weight Data

| MRID | Scientific Name | Rep | Weight | W Units |
|------|-----------------|-----|--------|---------|
| | Glycine max | 1 | 636 mg | |
| | Glycine max | 2 | 982 mg | |
| | Glycine max | 3 | 812 mg | |
| | Glycine max | | 0 | |

Records: 14 of 3

Records: 14 of 10

Appendix 5: Quality Control Testing Protocol Sample – Vegetative Vigor Database

The Quality Control Plan for the Vegetative Vigor Database (VVDB) is a multi-step process that integrates checking of various sources to verify the integrity of the data contained within the VVDB.

Definitions of what belongs in each data control box, or variable definitions, are included in the *Vegetative Vigor Database: Data Entry Key* document (Data Entry Doc). A successful check for the data entry tables is defined as an exact match between what is contained in the VVDB and the original source (i.e. USEPA approved website, expert EFED scientist, original study). An exact match entails matching numbers, letters or spelling, and wording as defined by the variable definitions. If the data for a particular field is not contained within the study, the field is left blank or contains a zero. This is also considered a valid match.

In some instances, a 100% check of the data entries is possible while in others 5% of the dataset is chosen for review due to the large number of records per table. The 5% will be selected using a random number generating program to create an unbiased subset of the data for analysis. Any errors found shall be corrected in the database immediately and documented on the printed out reviewer sheets. The error rate is determined by dividing the number of entries (not fields) containing errors by the total number entries checked.¹⁴ If the QC returns an error rate greater than 1%, the errors are to be corrected and the test is repeated. The test is to be carried out on another 5% from the remaining dataset and checked for errors until an error rate of less than 1% is achieved.

Chemical Name Table

The QC of the Chemical Name Table was completed on 8/10/06. All of the 64 entries were verified to be correct, indicating an error rate of 0% for all 192 fields.

Laboratory Names Table

The QC of the Laboratory Names Table was completed on 8/10/06. All of the 10 entries were verified to be correct, indicating an error rate of 0% for all 20 fields.

Species Names Table

The QC of the Species Names Table was completed on 8/10/06. All of the 30 entries were verified to be correct, indicating an error rate of 0% for all 180 fields.

Study Info Table

The QC of the Study Info Table was completed on 8/9/06 for the following studies as found using a random generator: 10, 32, 17, 48. No errors were found in the selected studies,

¹⁴ An entry is a single row of data containing multiple fields. For example, in the Chemical Name Table one entry is a chemical name and its associated CAS# and PC Code fields. If one of those fields is found to have an error, the whole entry is counted as faulty when determining the entry level error rate.

representing at least 5% of the study population. Through evaluating these 4 records and a totally of 112 fields, the error rate is projected to be 0% for the entire population.

Design Info Table

The QC of the Design Info Table was completed on 8/9/06 for the following studies as found using a random generator: 10, 33, 17, and 48. No errors were found in the selected studies, representing at least 5% of the study population. Through evaluating these 40 records and a totally of 520 fields, the error rate is projected to be 0% for the entire population.

Length Data Table

The QC of the Length Data Table was completed on 8/9/06 for the following studies as found using a random generator: 10, 33, 17, and 48. Two errors were found and corrected in the selected studies, representing at least 5% of the study population with an error rate of 0.29%. Through evaluating these 680 records and a totally of 3400 fields, the error rate is projected to be less than 1% for the entire population.

Weight Data Table

The QC of the Weight Data Table was completed on 8/9/06 for the following studies as found using a random generator: 10, 33, 17, and 48. Three errors were found and corrected in the 160 entries, representing at least 5% of the study population with an error rate of 1.88%. A second set of 5% was selected for review: 2, 7, 23, and 61. Evaluation of the weight data of these 4 studies found 2 errors in 400 fields, indicating an error rate of 0.5%. Through evaluating these 660 records and a totally of 3300 fields, the error rate is projected to be less than 1% for the entire population.

Photoperiod Table

The QC of the Photoperiod Table was completed on 8/9/06. Study numbers 32 and 4 were reviewed and no errors were found in the 22 entries or 154 fields, indicating an error rate of 0% for the entry dataset.

Light Intensity Table

The QC of the Photoperiod Table was completed on 8/9/06. Study numbers 32 and 48 were reviewed and no errors were found in the 24 entries or 240 fields, indicating an error rate of 0% for the entry dataset.

Environmental Conditions Table

The QC of the Environmental Conditions Table was completed on 8/9/06. Study numbers 43 and 48 were reviewed and no errors were found in the 37 entries or 481 fields, indicating an error rate of 0% for the entry dataset.

Study Info_Summary and Design Info_Summary Tables

The equations and data transfer for these sections is to be completed as soon as a separate EFED employee has the opportunity to go over the equations.